## REMARKS

Claims 1-6, 9-14 and 17-19 were pending in the present application. No amendments or cancellations have been made to the claims. No new matter has been added.

### Double Patenting Rejection of Claims 1-6, 9-14, and 17-19

Claims 1-6, 9-14, and 17-19 are rejected under the judicially created doctrine of nonstatutory obvious-type double patenting as being unpatentable over claims 1-17 of U.S. Patent No. 5,888,981. Applicants will submit an executed terminal disclaimer upon indication that the claims are allowable.

### Rejection of Claims 1, 6 and 9 under 35 U.S.C. § 112, First Paragraph

Claims 1, 6 and 9 are rejected under 35 U.S.C. § 112, first paragraph. The Examiner states that the instant specification "does not reasonably provide enablement for the claimed wherein the cell is present in a subject *in vivo* and one or both the nucleic acids are administered to a subject and the second nucleic acid is present in a cell." Applicants respectfully traverse this rejection.

Claim 1 is directed to a method for regulating expression of a *tet* operator-linked gene in a cell of a subject, comprising introducing into the cell a first nucleic acid molecule comprising the *tet* operator-linked gene, introducing into the cell a second nucleic acid molecule encoding a tetracycline-controllable transactivator (tTA), the tTA comprising a Tet repressor operably linked to a polypeptide which directly or indirectly activates transcription in eucaryotic cells, wherein the first and second nucleic acid molecules are not covalently linked to each other, and modulating the concentration of a tetracycline, or analogue thereof, in the subject. In one embodiment, the nucleic acid molecule encoding the tTA is introduced into the cell *ex vivo*, the method further comprising administering the cell to the subject. In another embodiment,

anhydrotetracycline, doxycycline or cyanotetracycline is used to modulate the activity of the tTA.

Applicants maintain that the specification provides ample guidance to enable one of ordinary skill in the art to make and use the invention, as described below and in the responses filed February 27, 2002 and February 21, 2003, incorporated herein. In the response filed on February 27, 2002, Applicants provided scientific publications as evidence that the claimed invention has been used successfully in *in vivo* experiments using different animal models, wherein gene expression was regulated using the claimed method in various tissue types via different delivery techniques. For example, previously submitted reference Dhawàn *et al.* describes use of the tTA regulatory system to successfully express a reporter gene in mouse skeletal muscle. Previously submitted reference Henninghausen also describes *in vivo* tTA regulated expression, wherein gene expression occurs in the secretory tissue and skin of mice. In another example, previously submitted reference Fishman *et al.* describes *in vivo* use of the tTA system with a tet operator-linked luciferase reporter gene, wherein expression is directed to the hearts of adult rats.

Furthermore, in the response filed on February 21, 2003, Applicants submitted additional post-filing references to support the position that the claimed method, wherein the tTA activator and the tet operator-linked gene of interest are found on nucleic acid molecule which are not covalently linked, has been used successfully *in vivo* using a variety of delivery techniques. For example, Applicants submitted reference Régulier *et al.* which describes the successful expression of CNTF (human ciliary neurotrophic factor) for improving neurologic conditions in experimental rats. Régulier *et al.* teach that expression of CNTF in rats can be achieved using the claimed methods, wherein the tTA and CNTF operatively linked to the tet operator are administered on two different viral vectors. Applicants also submitted reference Apparailly *et al.* which describes the

administration and transcriptional control of IL-10 using the claimed method for the treatment of rheumatoid arthritis using a mouse model. Each of the references previously submitted by Applicants describes the successful use of the claimed method for delivering and controlling a gene of interest in an *in vivo* animal model, wherein the non-covalently linked nucleic acids are administered to a specific tissue, including brain, intra-muscular, tumor, and airway, using a certain delivery methods, including injection into the target tissue and instillation.

The results described in the instant specification, including those presented in Example 2, in conjunction with the above citations, show the successful regulation of gene expression by the tTA system in multiple cell types *in vivo* using a variety of delivery techniques.

In response to Applicants' previous comments regarding the submitted references, the Examiner states that these references all "deliver[ed] DNA directly to the cells of interest" and that "[n]one of these articles supports enablement of administration by any route." The Examiner notes, however, that the specification is enabled for "a method of regulating expression of a tet operator-linked gene in a cell….wherein the method is carried out in a cell *in vitro* or *in vivo* wherein both the nucleic acids are introduced are administered directly to the cell."

As described above, Applicants teach a method for regulating expression of a *tet* operator-linked gene in a cell of a subject and provide working examples that the claimed invention works *in vivo*. Applicants teach at page 32, lines 28-29 that the nucleic acid molecules of the invention can be "introduced into the subject or the cells can be directly modified *in vivo* by convention techniques for introducing nucleic acid into cells." The Examiner has acknowledged that the specification is enabled for direct administration of the method of the claimed invention. Applicants have provided evidence that the claimed method is effective using a variety of conventional administration techniques, including

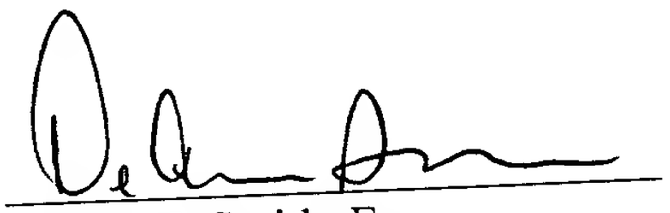
both injection and instillation (see previously submitted references Pulkkanen *et al.* and Rose *et al.*, respectively). *The administration techniques described in the previously submitted references demonstrate successful use of the claimed method. These references show that non-covalently linked nucleic acids of the claimed invention can be successfully delivered to tissue in a subject using a variety of methods known in the art,* e.g., adenoviral vectors (see Pulkkanen et al.), naked DNA (see Rose et al.), and lentiviral vectors (see Régulier et al.). Applicants respectfully submit that the Examiner has not provided evidence how these successful *in vivo* studies can be termed "direct" administration since they use different administrative techniques, e.g., injection or instillation, to target non-covalently linked nucleic acids to specific tissues, and further describe a variety of methods for targeting the nucleic acids to cells within the tissue. Applicants maintain that in view of the specification and the general knowledge in the art, evidenced by the previous reference submissions, the specification has provided sufficient guidance to the ordinarily skilled artisan to make and use the invention. Accordingly, Applicants respectfully request that the rejection of claims 1-6 and 9 under 35 U.S.C. §112, first paragraph, be withdrawn.

## SUMMARY

In view of the foregoing remarks, reconsideration of the rejections and allowance of all pending claims is respectfully requested.

If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the examiner is urged to call Applicants' Attorney at (617) 227-7400.

Respectfully submitted,



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